REMARKS

Claims 1-63 are pending in the subject application. Claims 1, 5-13, and 18-37 have been examined. By this Amendment claim 1 has been amended to recite only the elected invention as requested (Office Action, page 3, lines 1-3). Support for the amendment of claim 1 to recite "vesicular stomatitis virus" may be found, *inter alia,* in original claim 22. That amendment overcomes the indefiniteness rejection and one of the grounds of the enablement rejection, and renders the anticipation rejection moot. Claims 20-23 have been canceled, and claims 24-26 have been amended to correct their dependencies. Claim 34 has been amended to insert "and" prior to the last item in the Markush group. Accordingly the claims now under examination are claims 1, 5-13, 18-19, and 24-37.

Claims 2-4, 14-17, and 38-63 have been withdrawn from consideration as being drawn to nonelected inventions. The nonelected claims are being maintained of record pending the filing of one or more divisional applications directed to the subject matter thereof.

REFERENCE TO PRIOR APPLICATION

The application as filed contained a reference to the prior application, which was originally filed as a nonprovisional application and later converted to a provisional application in accordance with 37 CFR 1.53(c)(2). The reference as set forth in the subject application as filed omitted the Provisional Application number of the prior application because the Office had not yet rendered a Decision on applicants' Petition under 37 CFR 1.53(c)(2). On March 14, 2002 applicants amended the reference to the prior application to insert the Provisional Application number assigned to the prior application. Accordingly, the subject application complies with the requirements of 37 CFR 1.78(a).

CLAIMS ARE ENABLED

Claims 1, 5-13, and 18-37 have been rejected under 35 U.S.C. 112, first paragraph, on the grounds that "the specification, while being enabling for methods utilizing **VSV** for reducing the viability of mylogenous [sic] leukemia cell lines *in vitro*, does not reasonably provide enablement for the utilization of **all** viruses (that are not common human pathogens) for the reduction of viability of all hematopoietic tumor cells (either *in vivo* or *in vitro*). (Office Action, page 3) (emphasis in original). This rejection is respectfully traversed.

First, the rejection stated, "the specification is silent on the [sic] what other viruses other than VSV would induce the claimed antitumor effect." (Office Action, page 3). Because the claims recite that the virus "is a vesicular stomatitis virus", this ground of rejection is moot.

Second, the Office has improperly sought to place on applicants the burden of proving that the invention works. This is illustrated by the following passage from the rejection with respect to the tumor cells recited in the claims:

> "Additionally, the instant claims are drawn to **all** forms of hematopoietic tumor cells, while the specification has demonstrated only two leukemia cell lines (MD7E and L1210), a couple of AML cell lines OCI/AML3 and AML5, one CML cell line (K-562) and a T-cell leukemia (MOLT-4) that are that are susceptible to VSV infection."

Office Action, page 4 (bolding in original, underlining added). Contrary to the clear implication of the rejection, applicants are not required to submit experimental results demonstrating the anti-tumor activity of vesicular stomatitis virus. Rather, the Office bears the burden of establishing that the specification does not satisfy the enablement requirement of 35 U.S.C. §112, first paragraph. As stated by the CCPA in In re Marzocchi:

> "As a matter of Patent Office practice, then, a specification disclosure which contains a teaching of the manner and process of making and using the invention in terms which correspond in scope to those used in describing and defining the subject matter sought to be patented must be taken as in compliance with the enabling requirement of the first paragraph of § 112 unless there is reason to doubt the objective truth of the statements contained therein which must be relied on for enabling support."

In re Marzocchi, 439 F.2d 220, 223, 169 USPQ 367, ___ (underlining added). It is not sufficient for the Office to simply assert that it doubts the correctness of the statements in the disclosure. The Office must back up its doubts with evidence or reasoning. Again from In re Marzocchi:

> "In any event, it is incumbent upon the Patent Office, whenever a rejection on this basis is made, to explain why it doubts the truth or accuracy of any statement in a supporting disclosure and to back up assertions of its own with acceptable evidence or reasoning which is inconsistent with the contested statement."

In re Marzocchi, 439 F.2d at 224, 169 USPQ at ___ (internal citations omitted) (underlining added). No evidence or reasoning has been cited in support of the rejection. The mere insertion of the word "only" before a list of what applicants have shown experimentally does not qualify as acceptable evidence or reasoning to sustain an enablement rejection. To the contrary, the demonstrated success in a variety of hematopoietic tumor cells further supports applicants' position that the invention works for its intended purpose.

The Office has also improperly tried to place on applicants the burden of proving that the invention works *in vivo*. The rejection stated:

> "Claims 32-34 are drawn to the *in vivo* application of the claimed methods. People of skill in the art require documented evidence that a benefit can be derived by the therapeutic application of a given substance;

> however a survey of the relevant art does not indicate that substances such as those claimed provide such benefit."

Office Action, page 4. (To avoid possible confusion arising from the above-quoted passage in the Office Action applicants note that all of the claims encompass *in vivo* administration, not just claims 32-34.) This time the rejection has tried to shift the enablement burden to applicants by putting the Office's unsupported doubts in the mouth of unnamed, hypothetical "people of skill in the art" who are said to "require documented evidence that a benefit can be derived by the therapeutic application [i.e. *in vivo*] of a given substance." The unsupported citation to hypothetical "people of skill in the art" obscures such crucial issues as which people require such evidence, whether they are a minority or a majority of those of skill in the art, what evidence would they consider adequate, and the purpose for which they require such evidence. The purpose for which they allegedly require the evidence is necessary to avoid improperly importing into the enablement context the more stringent requirements under the Food and Drug Act for approval to market a therapeutic agent. It is, of course, important not to confuse "the requirements under the law for obtaining a patent with the requirements for obtaining government approval to market a particular drug for human consumption." In re Brana, 51 F.3d 1560, 1567, 34 USPQ2d 1436, ____ (Fed. Cir. 1995), citing, Scott v. Finney, 34 F.3d 1058, 1063, 32 USPQ2d 1115, 1120 (Fed. Cir. 1994). Applicants respectfully request that any allegations by the Office as to what people of skill in the art supposedly require be supported by literature citations or other evidence.

The allegation, quoted above, that "a survey of the relevant art does not indicate that substances such as those claimed provide such benefit" (Office Action, page 4) is wrong. As evidence that oncolytic viruses can have therapeutic effect *in vivo* applicants submit herewith a copy of Pecora, et al., J. Clin. Oncol. (2002) 20(9): 2251-2266 (Exhibit A), which reports the results of a phase I clinical trial that was sponsored by the assignee of the subject application. Despite being a phase I trial, Pecora et al. report that out of 62 assessable patients, "[e]vidence of efficacy included progression-free survival from 4 to

more than 30 months in 14 patients who had clear evidence of disease progression before initiation of [Newcastle disease virus therapy]." (Pecora, et al., page 2264, right column, first full paragraph).

The Office has attempted to require applicants to prove that the *in vitro* data contained in the specification correlate to an *in vivo* benefit. The rejection stated:

> "[T]he specification . . . does not provide any basis for correlating the *in vitro* results with beneficial effects that could reasonably be expected when said viruses are administered *in vivo* to 'treat' hematopoietic tumor cells, although *in vivo* use is clearly encompassed by the claims. Lacking either direct evidence for *in vivo* benefit, or a reasonable basis for correlating *in vitro* data as exemplified with *in vivo* benefit, the specification cannot be said to teach how to use the claimed viruses as pharmaceuticals."

Office Action, paragraph bridging pages 4-5. Applicants respectfully submit that it is accepted in the art to which this invention pertains that *in vitro* evidence of antitumor effect on tumor cell lines is reasonably correlated to *in vivo* therapeutic efficacy. The correlation is illustrated by the Pecora, et al. article (Exhibit A) discussed in the previous paragraph. It is also illustrated by U.S. Patent No. 5,677,178 (McCormick), which has been cited as allegedly anticipating certain claims of the subject application. The only experimental results contained in McCormick are the results of *in vitro* testing (Patent No. 5,677,178, column 18, line 42 to column 20, line 23; and Figures 2A-3C). Neverthless McCormick bases his teaching of human therapy on those *in vitro* results (Patent No. 5,677,178, column 16, line 45 to column 18, line 25). In contrast, the rejection has presented no evidence or reasoning to support its professed doubts as to the therapeutic efficacy of the claimed method.

Third, the Office has improperly sought to place on applicants the burden of explaining the mechanism by which the claimed invention works. This rejection stated, "The specification is silent on what receptor is utilized by VSV for cell entry." (Office Action,

page 4). As seen from the above-quoted passage, the Office has taken the position that the specification is required to explain how the viruses of the claimed invention enter the cells that they infect. That position is contrary to law because, "it is axiomatic that an inventor need not comprehend the scientific principles on which the practical effectiveness of his invention rests, nor is the inventor's theory or belief as to how his invention works a necessary element in the specification to satisfy the enablement requirement of 35 U.S.C. §112." Cross v. Iizuka, 753 F.2d 1040, 1042, 224 USPQ 739, ___ (Fed. Cir. 1985), citing Fromson v. Advance Offset Plate, Inc., 720 F.2d 1565, 1570, 219 USPQ 1137, 1140 (Fed. Cir. 1983).

Fourth, the Office has improperly sought to build an enablement rejection out of qualified statements in the specification concerning mechanism. The rejection stated, "The invention is predicated on the susceptible tumor cells lacking PKR activity, but the specification is silent on which hematopoietic tumor cells lack said function." (Office Action, page 4). Contrary to the above-quoted assertion from the rejection, applicants' invention is not limited to tumor cells that lack PKR activity. No such limitation appears in claim 1. The statement in the specification that "[p]referably the tumour cell lacks PKR activity" (Specification, page 4, lines 8-9) (underlining added) and the recitation of tumor cells lacking substantial PKR activity in dependent claims 18 and 19 are further evidence that the invention as a whole is not "predicated on the susceptible tumor cells lacking PKR activity". Understand the instant invention as being "predicated on the susceptible tumor cells lacking PKR activity" is all the more unreasonable in view of applicants' express warning that their discussion of PKR's possible role was being advanced "[w]ithout being bound by theory" (Specification, page 14, lines 14-18, esp. line 14).

Fifth, the Office has faulted the specification for being silent where it allegedly should not have been. The rejection stated:

> "[T]he specification is . . . silent on which interferon other than alpha interferon would provide normal cells protection from viral infection. . . . [T]he specification is . . . silent on how said viruses are to be administered to said subject."

Office Action, page 4. The reference to interferon presumably applies only to claims 24 and 37, which recite interferon. In accordance with this invention any interferon can be utilized. With respect to how the virus is administered to a subject, all conventional techniques and routes of *in vivo* administration can be utilized. The invention as claimed does not rest on the selection of certain types of interferon or (other than claim 32) certain routes of administration from among those known in the art.

In view of the foregoing, applicants respectfully submit that the enablement rejection is improper and should be withdrawn.

CLAIMS NOT INDEFINITE

Claims 1, 5-13, and 18-37 have been rejected as allegedly being indefinite. This rejection is respectfully traversed. The rejection sets forth five grounds of rejection, each of which is addressed by applicants in turn as follows:

1) The rejection stated that claim 1 is rendered vague and indefinite by the use of the phrase "common human pathogen". The amendment of claim 1 to delete the phrase "common human pathogen" overcomes this ground of rejection.

2) The rejection stated that claim 1 is rendered vague and indefinite by the use of the term "administering to the tumor cell a virus". The rejection stated that it "is unclear what is meant by said term. Is said virus 'injected' into said tumor cell or merely introduced into said cells [sic] environment?" (Office Action, page 5). In accordance with this invention, the virus can be administered to the tumor cell utilizing any

conventional technique. Of course, "breadth is not to be equated with indefiniteness." In re Miller, 441 F.2d 689, 693, 169 USPQ 597, ___ (CCPA 1971).

3) The rejection stated that claim 18 is rendered vague and indefinite by the use of the term "substantially no PKR activity". The rejection stated that it "is unclear what is meant by said term. At what level does PKR activity become 'substantially no' PKR activity?" (Office Action, page 5).

There is no requirement for applicants to define "substantially no" by means of a numerical cut-off. In the case of In re Marosi (710 F.2d 799, 218 USPQ 289 (Fed. Cir. 1983)) a claim reciting "essentially free of. . ." had been rejected as indefinite on the grounds that it was unclear "on this record where one skilled in the art would draw the 'essentially free of alkali metal' line between 4 ppm [parts per million] and 3,819 ppm.'" (Id. at 802). In reversing the rejection the CCPA stated, "Insofar as it requires appellants to specify a particular number as the cutoff between their invention and the prior art, the PTO's position is impractical." (Id. at 802).

Applicants maintain that the person of skill in the art would understand that the phrase "substantially no PKR activity" means a level of PKR activity no higher than what is considered insignificant in the art. Cf. Bausch & Lomb Inc. v. Alcon Laboratories Inc., 79 F.Supp.2d 243, 53 USPQ2d 1353 (W.D. N.Y. 1999). The term "substantially" is "ubiquitous in patent claims. Such usages . . . have been accepted in patent examination and upheld by the courts." Andrew Corp. v. Gabriel Electronics, Inc., 847 F.2d 819, 821, 6 USPQ2d 2010, ___ (Fed. Cir. 1988), cert. denied, 488 U.S. 927 (1988)

4) The rejection stated that claim 24 is rendered vague and indefinite by the use of the term "administering interferon to the tumor cell". The rejection stated that it "is unclear what is meant by said term. Is said virus 'injected' into said tumor cell or merely introduced into said cells [sic] environment?" (Office Action, page 6). In accordance with this invention, interferon can be administered to the tumor cell utilizing any

conventional technique. Again, "breadth is not to be equated with indefiniteness." In re Miller, 441 F.2d 689, 693, 169 USPQ 597, ____ (CCPA 1971).

5) In the "Markush" group recited in claim 34, the objected to "or" has been changed to -- and --.

CLAIMED INVENTION IS NOVEL

Claims 1, 5-7 and 11 have been rejected under 35 U.S.C. §102(b) as allegedly being anticipated by U.S. Patent No. 5,677,178 (McCormick). This rejection is respectfully traversed.

McCormick has been cited as teaching the use of "genetically reengineered adenoviruses to treat neoplasms" such as "lymphocytic leukemias" (Office Action, page 6). McCormick does not recite administration of a vesicular stomatitis virus. In contrast, claim 1 recites administration of a vesicular stomatitis virus, and claims 5-7 and 11 depend, directly or indirectly, from claim 1. Thus McCormick does not disclose all of the limitations of the claims. Therefore the rejection is improper and should be withdrawn.

INFORMATION DISCLOSURE STATEMENT

WO 99/18799, already of record, discloses anti-tumor activity of vesicular stomatitis virus. See page 9, lines 13-16; page 26, lines 1-4; and page 73.

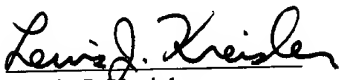
CONCLUSION

Reconsideration and withdrawal of all rejections and objections is respectfully requested.

It is believed that no fee is required in connection with the filing of this Amendment. If

any fee is required, the Commissioner is hereby authorized to charge the amount of such fee to Deposit Account No. 50-1677.

Respectfully submitted,



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MARKED-UP VERSION OF AMENDED CLAIMS

1. (Amended) A method of reducing the viability of a tumor cell, comprising administering to the tumor cell a virus, wherein said virus is [not a common human pathogen] a vesicular stomatitis virus and said tumor cell is [a carcinoma,] a hematopoietic tumor cell [, a glioblastoma, a melanoma, a sarcoma, or a neuroendocrine tumor].

24. (Amended) The method of claim [23] 7, further comprising administering interferon to the tumor cell prior to administering VSV.

25. (Amended) The method of claim [22] 5, wherein the virus is unable to inactivate PKR activity within the tumor cell.

26. (Amended) The method of claim [22] 5, wherein the virus is an attenuated strain of vesicular stomatitis virus.

34. (Amended) The method of claim 32, wherein the virus is contained in cell line infected with the virus and the administration comprises administering the virus-infected cell line to the subject by a route selected from intratumorally, intravenously [or] and intraperitoneally.

EXHIBIT A

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Phase I Trial of Intravenous Administration of PV701, an Oncolytic Virus, in Patients With Advanced Solid Cancers

By Andrew L. Pecora, Naiyer Rizvi, Gary I. Cohen, Neal J. Meropol, Daniel Sterman, John L. Marshall, Stuart Goldberg, Peter Gross, James D. O'Neil, William S. Groene, M. Scot Roberts, Harvey Rabin, Michael K. Bamat, and Robert M. Lorence

Purpose: **PV701, a replication-competent strain of Newcastle disease virus, causes regression of tumor xenografts after intravenous administration. This phase I study was designed to define the maximum-tolerated dose (MTD) and safety of single and multiple intravenous doses of PV701 as a single agent in patients with cancer.**

Patients and Methods: **Seventy-nine patients with advanced solid cancers that were unresponsive to standard therapy were enrolled. Four PV701 intravenous dosing regimens were evaluated: (1) single dose: one dose every 28 days; (2) repeat dose: three doses in 1 week every 28 days; (3) desensitizing: one lower dose followed by two higher doses in 1 week every 28 days; and (4) two week: one lower dose followed by five higher doses over 2 weeks every 21 days.**

Results: **A 100-fold dose intensification was achieved over 195 cycles. A first-dose MTD of 12×10^9 plaque-forming units (PFU)/m^2 was established for outpatient dosing. After an initial dose of 12×10^9 PFU/m^2, patients tolerated an MTD for subsequent doses of 120×10^9 PFU/m^2. The most common adverse events were flu-like symptoms that occurred principally after the first dose and were decreased in number and severity with each subsequent dose. Tumor site–specific adverse events and acute dosing reactions were also observed but not cumulative toxicity. Objective responses occurred at higher dose levels, and progression-free survival ranged from 4 to 31 months. Tumor tissue from one patient was obtained after 11 months of therapy and showed evidence of PV701 particles budding from the tumor cell membrane by electron microscopy and a pronounced lymphoplasmacytic infiltrate by histologic examination.**

Conclusion: **PV701 warrants further study as a novel therapeutic agent for cancer patients.**

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PV701[1,2] IS A HIGHLY purified, replication-competent, naturally attenuated strain of Newcastle disease virus,[3-6] an avian paramyxovirus. Newcastle disease virus strains, such as PV701, directly lyse diverse human cancer cells in vitro (oncolytic) while being significantly less toxic toward normal human cells.[1,3,7] Moreover, the virus is capable of both stimulating T-cell–mediated specific antitumor immunity and inducing nonspecific activation of immune function, such as the induction of cytokines (eg, interferon) and activation of tumoricidal macrophages.[8-10]

Newcastle disease virus is a rapidly replicating RNA virus with progeny virions first detectable in vitro within 3 hours after infection. After infecting a cancer cell, the virus rapidly spreads to neighboring tumor cells through the release of progeny virions and syncytia formation.[3,11] PV701 and certain other negative strand RNA viruses are selectively cytolytic for tumor cells as a result of defects in the interferon (IFN) signaling pathway that are common among diverse tumor types.[12,13] Defects in this pathway are believed to confer a growth and survival advantage to tumor cells.[13-16] However, these tumor defects also disable the antiviral function of IFN and confer sensitivity of malignant cells to infection and replication of viruses such as PV701.

Oncolytic Newcastle disease virus strains, including PV701, administered via intravenous, intraperitoneal, and intratumoral routes, replicate selectively in human cancer cells implanted in athymic mice, resulting in high rates of complete tumor regression and sparing of normal tissue.[2,3,17,18] In vitro death for most tumor cell lines occurs at a PV701 amount ~1,000-fold below the amount that adversely affects normal cells.[1] Similarly, there is an ~1,000-fold difference in the intravenous dose resulting in 50% tumor regression in athymic mice (~2×10^6 plaque-forming units [PFU]/mouse) and the median lethal dose (~5×10^9) in these mice.[2] In these animals, clear dose and dose frequency effects are observed, providing the rationale for examining such effects in the clinical setting. Objective

From the Cancer Center at Hackensack University Medical Center, Hackensack, NJ; Lombardi Cancer Center, Georgetown University Medical Center, Washington, DC; The Cancer Center at Greater Baltimore Medical Center, Baltimore, and Pro-Virus, Inc, Gaithersburg, MD; and Fox Chase Cancer Center and University of Pennsylvania Medical Center, Philadelphia, PA.

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responses (complete response and partial response) increase from ~50% to 100% by raising the dose three-fold or increasing the number of doses given at the same dose level from one to three doses.[2] In addition, intravenous exposure to a lower first dose of PV701 (3×10^8 PFU/mouse) results in a desensitization of the animal to toxicity from subsequent doses as evidenced by a 10-fold increase in the maximum-tolerated dose (MTD) for dose 2 (10^{10} PFU/mouse) compared with dose 1 (10^9 PFU/mouse). The oncolytic effect of PV701 requires live replication-competent virus because UV-inactivated virus caused no tumor regression.[2]

Oncolytic activity associated with Newcastle disease virus was first observed by Cassel and Garrett[19] when an intratumoral injection in a patient with cervical cancer resulted in tumor regression of the injected mass as well as a supraclavicular lymph node metastasis. Subsequent clinical trials of Newcastle disease virus focused primarily on a vaccine approach using viral oncolysates that included low doses of viable virus infecting autologous tumor cells.[8,20,21]

Characteristics of Newcastle disease virus that are favorable for human trials include the genetic stability of vaccine strains, the absence of genetic recombination, lack of a carrier state of naturally attenuated strains, and the lack of antigenic drift.[3] Human-to-human transmission has not been observed.[6] The virus has been safely given to humans in tumor vaccine studies, and accidental exposure has been reported to cause only self-limiting conjunctivitis.[3,5,6]

Other replication-competent viruses, including adenovirus and herpes simplex virus alone or in combination with chemotherapy, have caused tumor regressions in humans by the intratumoral route.[22-24] Early testing of both replication-competent and replication-incompetent adenoviruses by the intravenous route has been initiated,[25,26] but, until now, there has been no determination of the MTD for systemic therapy with a virus. Herein we report on a phase I study with dose escalations including the testing of various treatment schedules and MTD determination for systemic (intravenous) administration of the replication-competent virus PV701 in patients with advanced cancer that was unresponsive to standard therapy.

PATIENTS AND METHODS

Patient Enrollment

Seventy-nine patients were enrolled with advanced or metastatic solid malignancy that was unresponsive to treatment with established therapies. Entry criteria included at least one bidimensionally measurable tumor, ≥ 18 years of age, a life expectancy of at least 3 months, and a performance status (Eastern Cooperative Oncology Group) of 0 or 1. Laboratory result minimum entry requirements included 3,000 WBCs/μL, 1,500 neutrophils/μL, and 100,000 platelets/μL. Also required was a serum creatinine level less than 1.5 times the upper limit of normal, serum transaminase level less than 2.5 times the upper limit of normal (< 5.0 times the upper limit of normal for patients with metastatic liver disease), and a therapy-free period of 14 days. Patients were not eligible if documented to have CNS disease (including brain tumors on computed tomography [CT]/magnetic resonance imaging [MRI] scans required at screening), known hypersensitivity to eggs, antiviral or systemic corticosteroid treatment within 14 days, myocardial infarction or life-threatening arrhythmia within 6 months, known positivity for human immunodeficiency virus, active hepatitis B or C infection, an organ allograft, autoimmune disease, active viral infection (including cold or influenza), or uncontrolled bacterial infection. Active poultry workers and pregnant or nursing women were excluded. All patients provided informed written consent approved by the appropriate institutional review board.

PV701

PV701 is a highly purified isolate of the naturally attenuated (nonrecombinant) MK107 vaccine strain of Newcastle disease virus and is distinct from other strains, such as 73-T, that have been previously tested in humans. PV701 was cloned from MK107 by biologic means (triple-plaque purification in chicken embryo cells) to increase homogeneity and to remove defective particles. PV701 was grown to high titer in specific pathogen-free embryonated chicken eggs (SPAFAS, Inc, Preston, CT) and purified from allantoic fluid. Clinical lots of PV701 used in this study met release criteria, including potency, purity, sterility, and adventitious agent testing (Investigational New Drug Application BB 7401, May 7, 1998).

Plaque Assay

Doses were expressed as the amount of infectious virus in PFU. For the plaque assay, HT1080 human fibrosarcoma cells (obtained from American Type Culture Collection, Manassas, VA) were seeded into six-well tissue culture plates and grown to confluence. The growth medium was removed, monolayers were washed with serum-free medium, and 0.5 mL of various sample dilutions were added per well. The plates were incubated with rocking for 90 minutes at 37°C and 5% CO_2. The medium was completely removed, the monolayers were washed as above, and 3 mL of semisolid agar medium was overlaid onto each well. The cultures were incubated for 48 hours at 37°C and 5% CO_2. Monolayers were stained with neutral red for counting of plaques, and the virus titer was expressed as PFU/mL.

Intravenous Administration of PV701

For the first 47 patients, PV701 was prepared in a sterile syringe and patients were administered the dose over 10 minutes (regardless of dose level) by injection of the syringe contents into a port on a running intravenous line (at ~25 mL/h). For the subsequent 32 patients, PV701 was diluted into an intravenous saline bag (50 to 100 mL) immediately before dosing and administered at a rate of 1.2×10^9 PFU/m^2/min for doses of 12×10^9 PFU/m^2 and at a rate of 5.0×10^9 PFU/m^2/min for doses greater than 12×10^9 PFU/m^2.

Treatment and Study Design

Single-dose regimen. A single dose of 5.9, 12, or 24×10^9 PFU/m^2 was administered every 28 days (n = 17 patients). The starting dose of 5.9×10^9 PFU/m^2 was selected because this dose was greater than 1 log below the rodent MTD on the basis of body surface area. Dose

escalation proceeded by two-fold increments. All patients were hospitalized for 24 hours with intensive monitoring.

Repeat-dose regimen. Two dose levels were examined (n = 13 patients): either 5.9×10^9 PFU/m^2 or 12×10^9 PFU/m^2 was administered three times in 1 week every 28 days. Dose 2 was given 2 days after dose 1.

Desensitizing regimen. Five dose levels were examined (n = 37 patients): all patients received 12×10^9 PFU/m^2 (desensitizing dose) on the first day of administration followed by two doses of 24×10^9 PFU/m^2, two doses of 48×10^9 PFU/m^2, two doses of 72×10^9 PFU/m^2, two doses of 96×10^9 PFU/m^2, or two doses of 144×10^9 PFU/m^2. Dose 2 was given 2 days after dose 1. For each patient, all three doses were administered within 1 week and repeated every 28 days.

Two-week regimen. Two dose levels were examined (n = 12 patients): All patients received 12×10^9 PFU/m^2 (desensitizing dose) on the first day of administration followed by five doses of 96×10^9 PFU/m^2 or five doses of 120×10^9 PFU/m^2. Dose 2 was given 4 days after dose 1. Patients were given three doses per week for 2 weeks (six total doses) followed by 1 week off treatment. Enrollment was for a minimum of two courses.

General. Patients were monitored before each treatment and extensively after treatment. Evaluations included physical examinations, measurement of performance status, laboratory parameters, viral shedding (urine and sputum), and serum testing for PV701 antibodies and infectious virus. CT or MRI was used to assess tumor responses after each course of therapy.

A minimum of three patients were entered at each PV701 dose level until a patient experienced a dose-limiting toxicity (DLT). When a DLT was encountered, three additional patients were enrolled at that same dose level. There was no further dose escalation when two or more patients experienced a DLT. The MTD was defined as the dose level below that at which two or more DLTs were encountered.

Adverse events were graded using the Southwest Oncology Grading Scale. DLT was defined as a clinically significant adverse event (grade 4 leukocyte or neutrophil count lasting > 5 days; platelet count < 10,000/μL [grade 4 by NCI common toxicity criteria version 2.0]; or ≥grade 3 nonhematologic excluding fever and fatigue). Transient increases in hepatic transaminases (> grade 2) without grade 2 hyperbilirubinemia were not considered a DLT if these elevations returned to baseline before the next course. Symptoms clearly related to disease progression were not considered as DLTs.

All patients were eligible for additional courses of treatment when they had at least stable disease and an acceptable toxicity profile.

Virologic Studies

Samples of urine and sputum were screened for infectious virus by examining for cytopathogenic effects in cultures of HT1080 cells. For these screening assays of urine and sputum, spiked samples with as little as 100 PFU/mL and 100 PFU/g, respectively, were positive. All positive samples were quantified by plaque assay on HT1080 cells (as described in Plaque Assay section).

Neutralizing Antibody to PV701

Two-fold dilutions of heat-inactivated patient serum were mixed with a PV701 preparation that contained 3×10^2 PFU/mL in a total volume of 2 mL. After incubation for 1 hour at room temperature, 0.5 mL of the serum-virus mixture was tested for infectivity using the plaque assay described above. The neutralizing antibody titer of the serum sample is expressed as the last dilution resulting in at least 80% reduction in the number of plaques.

Cytokine Measurements

Patient serum was analyzed using commercially available enzyme-linked immunosorbent assay kits for detection of human cytokines (IFN-α and IFN-γ, BioSource International, Camarillo, CA; IFN-β, Fujirebio, Inc, Tokyo, Japan; interleukin-6 [IL-6] and tumor necrosis factor-alpha [TNF-α], Pierce-Endogen, Rockford, IL). Sera from 10 patients were analyzed including two patients who were given a single dose of 12×10^9 PFU/m^2, one patient who was given a single dose of 24×10^9 PFU/m^2, five patients who were given three repeat doses of 12×10^9 PFU/m^2, and two patients in the desensitizing regimen who were given a first dose of 12×10^9 PFU/m^2 followed by two doses of 24×10^9 PFU/m^2.

Tissue Processing

Sections of formalin-fixed patient tissues were processed for hematoxylin and eosin (H&E) staining. Electron microscopy of patient tissue samples was performed by negative staining with uranyl acetate and compared with those from experimental HT1080 human fibrosarcoma xenografts infected with PV701.[2]

Statistical Analysis

Assessments of the association between age or baseline anemia and grade 3 flu-like symptoms, the association of transaminase elevations (> 200 U/L) and preexisting liver metastases, and the association of preexisting lung disease and oxygen desaturation were performed using Fisher's exact test. The null hypothesis was that the probability of an adverse event was the same in patients with and without the baseline characteristic. A two-sided alternative was considered statistically significant at $P < .05$.

RESULTS

Patient Characteristics

Seventy-nine patients (48 men and 31 women) with advanced cancer that was unresponsive to standard therapy were enrolled onto this study (Tables 1 and 2) from June 1998 through September 1999 and were treated over 12 dose levels with a total of 195 cycles. The median age was 58 years (range, 24 to 81 years) with 22 patients (28%) older than 70 years. The most common primary tumor types were colorectal (n = 23), pancreatic (n = 9), renal (n = 9), breast (n = 8), and non–small-cell lung carcinoma (n = 8). Seventy-two patients had received previous chemotherapy; 35 of them received three or more regimens.

Antibody Response

Thirty-two patients were tested at baseline for neutralizing antibody to PV701. One of these patients was positive at the limit of detection of the assay. His adverse event profile was no different from that of other study subjects. The other 31 patients tested all were negative for neutralizing antibody.

Table 1. Dosage Levels

Dose Level	Regimen	Doses ($\times 10^9$ PFU/m^2)	Dose Intensity ($\times 10^9$ PFU/m^2/course)	No. of Patients Enrolled	No. of Cycles
1	Single	5.9	5.9	6	19
2	Single	12	12	6	11
3	Single	24	24	5	13
4	Repeat	5.9 × 3	17.7	6	8
5	Repeat	12 × 3	36	7	11
6	Desensitizing	12, 24 × 2	60	4	10
7	Desensitizing	12, 48 × 2	108	3	37*
8	Desensitizing	12, 72 × 2	156	5	14
9	Desensitizing	12, 96 × 2	204	12	28
10	Desensitizing	12, 144 × 2	300	13	13
11	Two-week	12, 96 × 5	492	7	18
12	Two-week	12, 120 × 5	612	5	13
Total				79	195

*This cohort includes patient no. 521, who received 34 cycles of PV701 including his most recent 25 cycles at 12/120 × 5 (10^9 PFU/m^2).

Fourteen of 16 patients in the single-dose regimen, seven of seven patients in the repeat-dose regimen, and six of six patients in the desensitizing-dose regimen became seropositive, first evident 1 to 2 weeks after PV701 dosing. By week 4 after initial dosing, 10 of 12 patients tested in the repeat-dose and desensitizing regimens had neutralizing antibody titers at 1:320 to 1:640. Eight patients were tested at 5 to 10 weeks after initial dosing, and they had a median neutralizing antibody titer of 1:640 (range, 1:80 to 1:2,560). One patient was followed over 18 courses (1.5 years). At month 3, his titer reached a plateau (at 1:2,560) that has persisted through the last time point analyzed (month 18).

Virology

A total of 821 sputum samples and 899 urine samples from 67 patients were examined for virus shedding using highly sensitive infectivity assays. Positive samples were quantified by plaque assay. Fewer than 1% of the sputum samples tested were positive. These positive samples contained low PV701 levels (median 26 PFU/g sputum). No PV701 was detectable in the sputum at day 14 or beyond. All repeat course sputum samples were negative. Fifteen percent of all urine samples tested positive at a low level (median, 820 PFU/mL), and all samples were negative 3 weeks after the last dose. Five percent of urine samples analyzed from courses 2 to 6 were positive for PV701. The percentage of patients with transient viruria at any time during a course dropped from a first course high of 54% to 0% in patients who received seven or more courses of PV701.

Cytokines

Serum samples from 10 representative patients who were given one or multiple doses (12 or 24 × 10^9 PFU/m^2) were analyzed at multiple time points for serum proinflammatory cytokines (IFN-α, IFN-β, IFN-γ, IL-6, and TNF-α). Similar patterns of cytokine production were noted for all 10 patients after their first dose of PV701. IFN-α was the predominant cytokine produced in all patients (median peak levels, 20,000 pg/mL) compared with the four other cytokines (median peak levels between 10 and 200 pg/mL). Detectable increases in IFN-α, IFN-γ, IL-6, and TNF-α were first seen by 6 hours after dosing with IFN-β only detectable at 20 hours after dosing. All four cytokines consistently reached peak levels at 20 hours after dosing and returned to or near baseline by 2 to 3 days after dosing. In patients who received more than one dose of PV701, there was a marked reduction in serum cytokine levels after the second dose compared with the levels seen after the first dose (eg, for IFN-α, a median peak of 13,000 pg/mL noted after the first dose compared with a median peak of 65 pg/mL after the second dose).

Toxicity

Most common adverse events. Throughout the trial, fever, other cytokine-related flu-like symptoms (eg, chills, fatigue, nausea/vomiting, headache, diarrhea), and hypotension were the most common adverse events (Tables 3 and 4), primarily occurring 4 to 24 hours after PV701 dosing. Except for the immediate dosing reactions (detailed below), adverse events diminished markedly in number and severity with repeat dosing (Table 4) and with the second (see Table 3) and all subsequent courses.

Two of the first three patients in the first cohort (5.9 × 10^9 PFU/m^2) had grade 3 fever of 40.0°C to 40.6°C, which was promptly reversed with ibuprofen. Beginning with the fifth patient in this cohort, all subsequent patients in this trial received acetaminophen and ibuprofen prophylaxis and the

Table 2. Patient Characteristics

		No. of Patients
Total No.		79
Age, years		
Median	59	
Range	24-81	
Male:female		48:31
Primary tumor site		
Colorectal		23
Pancreatic		9
Renal		9
Breast		8
Non–small-cell lung		8
Sarcoma		4
Head and neck		4
Melanoma		3
Mesothelioma		2
Esophageal		2
Lymphoma		2
Ovarian		1
Bladder		1
Carcinoma (unknown primary)		1
Cholangiocarcinoma		1
Carcinoid		1
No. of prior chemotherapy regimens		
0		7
1		13
2		24
≥ 3		35
No. of prior immunotherapy regimens		
0		60
1		9
2		10
No. of prior hormonal therapy regimens		
0		69
1		2
≥ 2		8
No. of prior investigational agents		
0		61
1		11
≥ 2		7
Prior surgery		68
Prior radiation therapy		40

incidence of grade 3 fever was reduced to 11% (eight of 75 patients).

In the single-dose regimen, 42% of patients (seven of 17) had at least one episode of diarrhea including one case of grade 4. In subsequent dosing regimens, diarrhea was effectively controlled using loperamide with 10% of patients having diarrhea.

Age and baseline anemia were examined in all 79 patients as potential risk factors for grade 3 flu-like symptoms (fever, fatigue, nausea, vomiting, and dehydration). Age was examined because two DLTs at the 12/144/144 $\times 10^9$ billion PFU/m^2 dose level occurred in elderly patients (81 and 75 years of age; see Dose Escalations, DLT, and Determination of MTD below). Anemia was examined as a risk factor because it might exacerbate the severity of any fatigue, the most common of the grade 3 flu-like symptoms. The analysis showed that 13 (59%) of 22 patients ≥ 70 years of age had grade 3 flu-like symptoms compared with 18 (32%) of 57 less than 70 years of age ($P < .025$), and 24 (52%) of 46 patients with baseline anemia (Hgb < 11 or Hct ≤ 35) had grade 3 flu-like symptoms compared with seven (21%) of 33 patients without baseline anemia ($P < .01$).

Desensitization to toxicity on repeat dosing. As predicted from the animal models, dose 1 desensitized patients to the flu-like symptoms on subsequent doses. Table 4 lists the six most common adverse events observed for patients in the desensitizing regimen in order of decreasing incidence and by the Southwest Oncology Group severity grade for each dose. Adverse events were reduced in number and severity after the second and third doses despite a two-fold to eight-fold increase in dose. With all patients receiving prophylactic antipyretics, the incidence of grade 3 fever for patients in this regimen was reduced from 13% on dose 1 to being undetected with subsequent doses (Table 4). The incidence of grade 1 to 2 fever reduced from 83% with dose 1 to 17% with dose 3. A similar pattern of desensitization to adverse events was seen in the 2-week dosing regimen with doses 2 to 6 producing milder and less frequent adverse events compared with dose 1, even when doses 2 to 6 were eight- to 10-fold higher (data not shown). This desensitization to toxicity with repeat doses was also seen for the hematologic changes (see Hematology/Coagulation Profiles below).

Acute dosing reactions. Acute and reversible dosing reactions were observed in five of the first seven patients enrolled at the 12/96/96 $\times 10^9$ PFU/m^2 dose level, typically during the third dose of the first course. These reactions consisted of back pain, chest tightness, chest pain, and hypertension. Abdominal pain was less commonly seen. In all cases, the onset was within 5 minutes of the start of dosing and resolved spontaneously and completely within 30 minutes of the beginning of the adverse event. In a few instances, these adverse events required a pause in the administration of virus. One patient experienced grade 3 back pain on his third PV701 injection and was the only patient in the study who did not complete a PV701 dosing because of this acute dosing reaction. All other acute dosing reactions were grade 1 or 2. These reactions were attributed to the rate of administration of the virus, which had increased from 1.2 $\times 10^9$ PFU/m^2/min at the 12 $\times 10^9$ PFU/m^2 dose level to 1.0 $\times 10^{10}$ PFU/m^2/min at the higher dose levels. In subsequent patients, the administration rate for doses above 12 $\times 10^9$ PFU/m^2 was decreased to 5 $\times 10^9$

Table 3. Adverse Events for All Patients During the First Two PV701 Courses

Adverse Event	Course 1 (N = 79)			Course 2 (n = 39)		
	Grade 1/2 (%)	Grade 3 (%)	Grade 4 (%)	Grade 1/2 (%)	Grade 3 (%)	Grade 4
Flu-like symptoms						
Fever	92	13	–	59	3	–
Chills	73	–	–	44	–	–
Fatigue/malaise	70	32	–	26	3	–
Headache	34	1	–	5	–	–
Myalgia	23	–	–	13	–	–
Diaphoresis	19	–	–	13	–	–
Gastrointestinal						
Nausea	72	1	1	26	–	–
Vomiting	57	4	1	23	–	–
Anorexia	54	–	–	18	–	–
Diarrhea	53	1	3	15	–	–
Constipation	16	1	–	5	–	–
Dehydration	15	9	–	–	–	–
Dry Mouth	10	–	–	–	–	–
Hematologic						
Thrombocytopenia	46	13	3*	–	–	–
Anemia	38	8	1	13	3	–
Leukopenia	37	23	4†	18	–	–
Increased PT/PTT	30	1	1	15	–	–
Neutropenia	23	8	1	8	–	–
Cardiovascular						
Hypotension	52	5	1	15	–	–
Edema, peripheral	15	3	–	13	–	–
Dizziness	14	–	–	–	–	–
Respiratory						
Dyspnea/hypoxia	27	8	6	15	3	–
Cough	18	–	–	5	–	–
Liver						
Increased ALT/AST	47	18	4	18	3	–
Increased bilirubin	4	10	3	–	–	–
Neurologic						
Pain, back/flank	24	8‡	–	15	3	–
Pain, abdominal	24	4	–	18	3	–
Confusion/disorientation	18	3	–	–	–	–
Anxiety/agitation	13	–	–	5	–	–
Pain, hip/leg	10	–	–	13	–	–
Acute dosing reaction						
Back pain	19	1	–	26	–	–
Chest pain	11	1	–	18	–	–
Metabolic						
Hypokalemia	10	–	–	5	–	–

NOTE. Included are all events with at least a 10% incidence for any grade.

*For both cases of SWOG grade 4 thrombocytopenia (platelet nadir at 23,000/μL in a patient dosed with a single dose of 24×10^9 PFU/m^2 and platelet nadir at 19,000/μL in a patient dosed at 12/96/96 $\times 10^9$ PFU/m^2), platelets had recovered to a grade 1 to 2 level within 4 to 6 days and no bleeding was observed.

†All three cases of grade 4 leukopenia occurred 20 hours after dosing and had recovered to grade 2 at the next time point (3 days later).

‡Grade 3 pain occurred in patients with baseline pain at this location.

PFU/m^2/min (see Patients and Methods). At the slower administration rate, dosing reactions occurred infrequently in subsequent patients and were less severe. The symptoms of back pain, chest tightness, and hypertension were suggestive of a vasospasm effect, although no ECG changes were observed and prophylaxis with antihistamines was found to be ineffective.

Tumor site–specific adverse events including inflammation. A separate class of adverse events dependent on the tumor location was noted. These tumor site–specific adverse events included the following:

- Tumor inflammation/edema on physical examination (one patient with a scalp metastasis from a colon carcinoma, the other with tongue carcinoma).

Table 4. Percentage of the Six Most Common Adverse Events Observed for Patients Given Three PV701 Doses for Which Doses 2 and 3 Were Up to Eight-Fold Higher Than Dose 1 (n = 24 patients, desensitizing regimen)

Adverse Event	Dose 1 (n = 24)		Dose 2 (n = 23)		Dose 3 (n = 23)		Dose 1, Course 2 (n = 14)	
	Grade 1-2 (%)	Grade 3 (%)	Grade 1-2 (%)	Grade 3 (%)	Grade 1-2 (%)	Grade 3 (%)	Grade 1-2 (%)	Grade 3 (%)
Fever	83	13	82	0	17	0	27	0
Chills	54	0	39	0	17	0	20	0
Nausea	46	8	22	0	30	0	13	0
Fatigue	41	38	21	4	26	9	7	0
Vomiting	38	8*	4	0	17	0	7	0
Hypotension	33	0	26	0	4	0	0	0

NOTE. Includes all patients at dose levels 12/24/24 (four patients), 12/48/48 (three patients), 12/72/72 (five patients), and 12/96/96 × 10^9 PFU/m^2 (12 patients).

*In addition, there was one case of grade 4 vomiting.

- Oxygen desaturation observed only in patients with lung/pleural tumor involvement (13 of 55 patients with pulmonary tumor involvement *v* zero of 24 without involvement; $P < .01$).
- Pulmonary adverse events (grade ≥ 3, including six cases of grade 3 dyspnea) in nine of 55 patients with pulmonary tumor involvement. Seven of these nine patients had one or more of the following baseline characteristics (signs, symptoms, radiographic evidence): baseline grade 2 dyspnea, lung tumors at least 5 cm in size, significant pleural effusions, partial or complete lobectomy, lobar atelectasis, and lobar consolidation. There were no clinically significant pulmonary adverse events observed in the 24 patients without lung involvement by tumor.
- Transiently elevated liver transaminases over 200 U/L occurred only in patients with liver metastases (18 of 38 patients with liver metastases *v* zero of 41 patients without; $P < .01$).
- An enterocutaneous fistula at the tumor site (with the tumor extending from bowel to skin surface) in a 63-year-old man with a colon carcinoma occurred 9 days after his first dose of 144 × 10^9 PFU/m^2.

Hematology/coagulation profiles. After the first dose of the first course of PV701, all patients experienced a transient drop in leukocyte and platelet counts with full recovery to baseline observed within 7 to 14 days, regardless of dose level. Clinically significant thrombocytopenia (Southwest Oncology Group grade 4, nadirs of 19,000 and 23,000 platelets/μL) and leukopenia (grade 4) were observed in two and three patients, respectively. However, these patients were carefully monitored clinically with follow-up hematology profiles performed 12 hours to 4 days later and a rapid recovery in blood counts was observed in all cases. No episodes of bleeding or infection resulted from these transient drops in counts. The pattern of thrombocytopenia and leukopenia was identical for patients who were given one dose as for those who were given up to six doses, indicating that this phenomenon was due to the first PV701 dose. The rate of recovery was the same for the single-dose patients as for those who were given multiple PV701 doses with full recovery noted by day 14 in all patients, including those who were given subsequent doses up to 10 times higher than dose 1. There were no significant changes in leukocyte and platelet counts during subsequent courses.

There were no significant changes in hemoglobin or hematocrit values after PV701 dosing in patients without baseline anemia. Grade 3 anemia was reported for six patients, all of whom had significant anemia at baseline.

Specific assays were added to the standard coagulation panel to serve as early predictors of potential disseminated intravascular coagulation (ie, fibrinogen and fibrin split products). There were no dose- or time-related changes in these parameters or in the standard coagulation parameters (prothrombin time, partial thromboplastin time) that were considered clinically significant and related to therapy.

Hypoglycemia in patients on oral hypoglycemic agents or insulin. Three instances of clinically significant hypoglycemia occurred. All three patients had diabetes (two were receiving oral hypoglycemic agents, and one was receiving insulin). After the initial PV701 dose, these patients experienced nausea and dehydration, resulting in limited oral intake. Hypoglycemia was not observed after subsequent PV701 dosing. It is unknown whether PV701 administration also increased the bioavailability of the hypoglycemic therapy. Discontinuing these agents in the immediate postdosing period after dose 1 resulted in no additional episodes of hypoglycemia.

Dose escalations, DLT, and determination of MTD. In the single-dose regimen, doses between cohorts were escalated in two-fold increments from 5.9 to 24 × 10^9 PFU/m^2. As can be seen in Table 5, one adverse event (grade 4

Table 5. Dose-Limiting Toxicities

Dose Level	Regimen	Doses ($\times 10^9$ PFU/m^2)	Dose Being Escalated	No. of Patients Enrolled	No. of Patients With DLT	Type of DLT
1	Single	5.9	First dose	6	1	1 pt with diarrhea (gr 4)
2	Single	12	First dose	6	0	None
3	Single	24	First dose	5	1*	1 pt with dyspnea† (gr 3) and hypoglycemia (gr 3)
4	Repeat	5.9 × 3	N/A‡	6	1	1 pt with dyspnea§ (gr 4)
5	Repeat	12 × 3	N/A‡	7	0	None
6	Desensitizing	12, 24× 2	Second dose	4	0	None
7	Desensitizing	12, 48 × 2	Second dose	3	0	None
8	Desensitizing	12, 72 × 2	Second dose	5	0	None
9	Desensitizing	12, 96 × 2	Second dose	12	1	1 pt with acute dosing reaction‖ (gr 3 back pain)
10	Desensitizing	12, 144 × 2	Second dose	13	3¶	1 pt with tremors (gr 3) and dehydration (gr 3); 1 pt with dehydration (gr 3); 1 pt with hypoxia (gr 3) which occurred during rigors
11	Two-week	12, 96 × 5	N/A‡	7	0	None
12	Two-week	12, 120 × 5	N/A‡	5	0	None#

Abbreviations: DLT, dose-limiting toxicity; pt, patient; gr, grade; N/A, not applicable.

*Grade 2 hypotension was also observed at this dose level (in three of five patients) and was the only dose-dependent toxicity in the single-dose regimen. Dose escalation was stopped to allow an outpatient dosing regimen. The dose of 12 × 10^9 PFU/m^2 was therefore established as the outpatient MTD for the first dose with grade 2 hypotension as dose limiting.

†This patient with baseline compromised lung function had worsening of underlying pulmonary infiltrate after PV701 dosing. See the Results, which describes an association of lung/pleural tumor involvement and respiratory adverse events.

‡Tolerance of repeat dosing tested rather than dose escalation.

§This patient with baseline extensive lung metastases and bilateral pleural effusions experienced grade 4 dyspnea after the first dose of 5.9 × 10^9 PFU/m^2 and did not experience any recurrence with doses 2 and 3 or during a second course.

‖One patient of the first seven of this cohort had an acute dosing reaction (grade 3 back pain) due to the 96 × 10^9 PFU/m^2 dose. The infusion rate was slowed and five more patients enrolled onto this dose level and only one mild dosing reaction occurred (grade 1 abdominal discomfort).

¶Dose escalation of the second dose was stopped at 144 × 10^9 PFU/m^2 due to the occurrence of three dose-limiting toxicities in this cohort.

#One patient with preexisting compromised lung function died of respiratory failure after receiving only the desensitizing dose of 12 × 10^9 PFU/m^2. There were no dose-limiting toxicities seen with the dose of 120 × 10^9 PFU/m^2.

diarrhea) that met the definition of DLT was seen in the first cohort (5.9 × 10^9 PFU/m^2) of six patients. Severe diarrhea was not seen on subsequent higher dose levels when loperamide was given prophylactically at the first sign of gastrointestinal side effects. No DLTs were seen in six patients in the 12 × 10^9 PFU/m^2 cohort. At the 24 × 10^9 PFU/m^2 dose level, a DLT (grade 3 dyspnea) occurred in a patient with a lung tumor mass and baseline signs of pulmonary infiltrate. This patient also experienced grade 3 hypoglycemia. Dyspnea and hypoglycemia were not considered dose dependent in this trial because severe dyspnea was associated with patients having lung/pleural tumor masses and hypoglycemia only occurred in patients with diabetes (discussed in Hypoglycemia in Patients on Oral Hypoglycemic Agents or Insulin).

In the single-dose regimen, the only dose-dependent toxicity was grade 2 hypotension, which occurred in three of five patients in the 24 × 10^9 PFU/m^2 cohort. Because the intention was to establish an outpatient dosing regimen, dose 1 was not escalated further. The dose of 12 × 10^9 PFU/m^2 was therefore established as the outpatient MTD for the first dose with grade 2 hypotension as dose limiting.

The repeat-dose regimen tested for the presence of any cumulative toxicity at two dose levels (5.9/5.9/5.9 and 12/12/12 × 10^9 PFU/m^2) in a total of 13 patients. As indicated in Table 5, only a single DLT was observed (grade 3 dyspnea), which occurred in a breast cancer patient with baseline bilateral pleural effusions dosed at 5.9/5.9/5.9 × 10^9 PFU/m^2, the lower of the two dose levels. No cumulative toxicity was seen. A dose of 12 × 10^9 PFU/m^2 was therefore chosen as a first dose (or "desensitizing dose") for escalation of the second and subsequent doses in the desensitizing regimen.

In the desensitizing regimen, one DLT was observed in the first four cohorts with a total of 24 patients (Table 5). This acute dosing reaction (grade 3 back pain) at 12/96/96 was attributed to the infusion rate (see Acute Dosing Reactions above), which was slowed for subsequent patients. In the 12/144/144 × 10^9 PFU/m^2 cohort of 13 patients, three DLTs were observed and dose escalation was

stopped. These events were seen after the 144 × 10^9 PFU/m^2 dose: grade 3 tremors and dehydration in an 81-year-old woman, grade 3 dehydration in a 75-year-old man, and grade 3 hypoxia associated with 30 minutes of rigors in a man with lung cancer.

For patients in the 2-week regimen, repeat doses lower than 144 × 10^9 PFU/m^2 were therefore tested. There was no significant difference in adverse event profile or laboratory values for repeat doses of 96 or 120 × 10^9 PFU/m^2 given five times over 2 weeks, no cumulative toxicity was seen, and patients tolerated equally well either of these doses. Therefore, the dose of 120 × 10^9 PFU/m^2 was determined to be the second dose MTD.

Serious Adverse Events and Deaths

Seven cases of dehydration requiring intravenous fluids and/or hospitalization were noted. Most of these cases were associated with significant nausea/vomiting and/or diarrhea. These events were reversible and did not result in lasting effects. As discussed above, patients over age 70 were at increased risk for flu-like grade 3 adverse events.

Three patients with baseline bacterial infections were administered PV701 and had episodes of sepsis after PV701 therapy. Two patients had a baseline urinary tract infection. The other patient had baseline fever in the week before beginning PV701 treatment and had baseline bacteremia immediately before his first dose of PV701.

There were five patient deaths, four of which were clearly attributed to progressive disease occurring during the 4-week reporting period. The remaining death occurred in a 55-year-old man with renal carcinoma metastatic to the lungs. At baseline, he had compromised pulmonary function as a result of previous lobectomies, lobar atelectasis, and an 8-cm metastasis in one of the two remaining lobes. In addition, he was status postradical nephrectomy with a 4-cm tumor metastasis in his remaining adrenal gland. This patient was enrolled in the 12/120 × 5 doses x 10^9 PFU/m^2 dose level. After an initial (and only) PV701 dose of 12 × 10^9 PFU/m^2, he experienced grade 3 hypotension that required intravenous hydration and 48 hours of hospitalization. Three days later, he was admitted to a local community hospital with complaints of fatigue, lethargy, and severe respiratory distress. Mechanical ventilation was advised but was declined. The patient died as a result of respiratory failure approximately 12 hours later. At autopsy, an enlarged subcarinal lymph node (5 × 4 × 3 cm) filled with partially hemorrhagic and necrotic tumor tissue was reported as well as a metastatic tumor that measured 8 × 7 × 6 cm just below the inferior pleural surface of the left upper pulmonary lobe. Histologic sections of the left lung were reported as showing the presence of localized thrombi only in the tumor vessels with tumor necrosis and severe edema/inflammation only in the tumor-bearing lung and mild to absent in the non–tumor-bearing lung. Inflammation was not reported in any other organ.

Response Assessment

Seventeen patients were not eligible for response assessment. Twelve patients were removed from the study before a radiographic response assessment because of toxicity or worsening baseline symptoms. Three patients were taken off the study for progressive disease. One patient was removed as a result of noncompliance, and one was removed to receive radiation therapy.

Of the 62 patients who were eligible for response assessment, 14 had freedom from tumor progression for 4 to 30+ months and two had radiographic evidence of major responses. A complete response was documented in a 51-year-old man with tonsillar (squamous cell) carcinoma. At the time of enrollment, this patient had disease progression during cisplatin and radiation therapy (with the most recent treatment given in September 1998) as noted by a radiographic increase during the preceding 3 months. After a baseline MRI scan in January 1999 (Fig 1A) demonstrating a 1.5-cm tumor in the pharynx, he received PV701 at the 12/96/96 × 10^9 PFU/m^2 dose level. After one cycle, he achieved a radiographic complete response as evidenced by resolution of the tumor on MRI. Follow-up scans after months 2, 3, and 5 of therapy confirmed the radiographic complete response (Fig 1B). The patient was noncompliant and discontinued therapy between months 5 and 7. An MRI scan at month 7 indicated disease progression elsewhere in the pharynx (lateral oropharyngeal wall).

A partial response was documented in a 79-year-old-man who had colon carcinoma and had failed capecitabine, 5-FU, and irinotecan. He had not received any chemotherapy in the 2 months before his enrollment into the PV701 study at the 12/72/72 × 10^9 PFU/m^2 dose level. At baseline, he had two liver metastases, the largest one well-circumscribed and measuring 10 cm in maximal dimension (Fig 2A). His CT scans at month 1 (Fig 2B) and month 2 after therapy showed overall tumor regression of greater than 70%. In addition, immediately after dosing there was a spike in carcinoembryonic antigen level (approximately 4.5-fold increase) followed by a drop to steady-state levels at 79% below baseline, shown elsewhere to be further indicative of a response.[27] Progression-free survival of 10 months was observed in this patient. Seven other patients with diverse malignancies (including mesothelioma, melanoma, colon carcinoma, breast carcinoma, pancreatic carcinoma, and carcinoid) had measurable tumor reduction, although less than 50% of the total tumor burden.

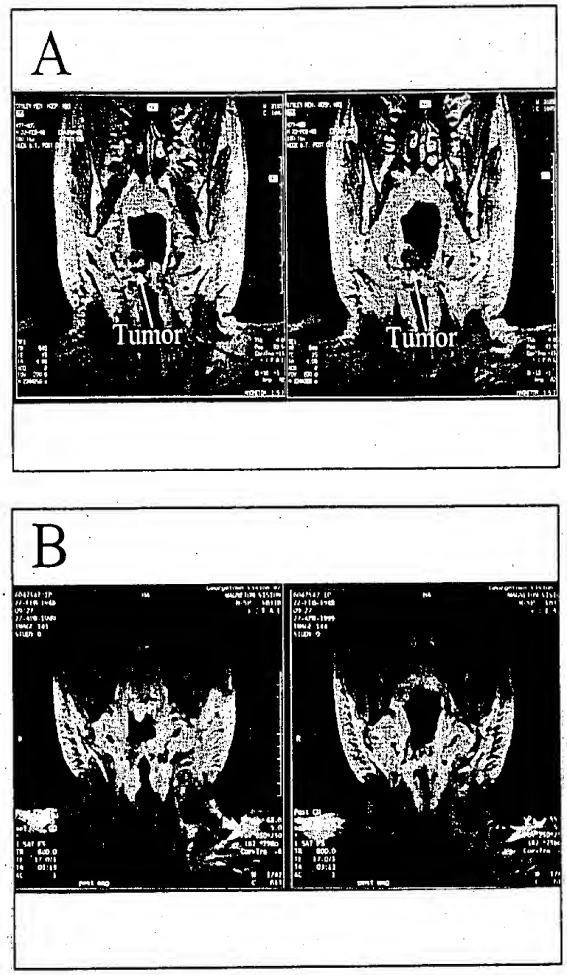

Fig 1. Complete response in a man with tonsillar carcinoma. (A) Baseline MRI image showing a 1.5-cm tumor at the junction of the right tongue base and tonsillar pillar. (B) MRI image at month 3 showing complete resolution of tumor.

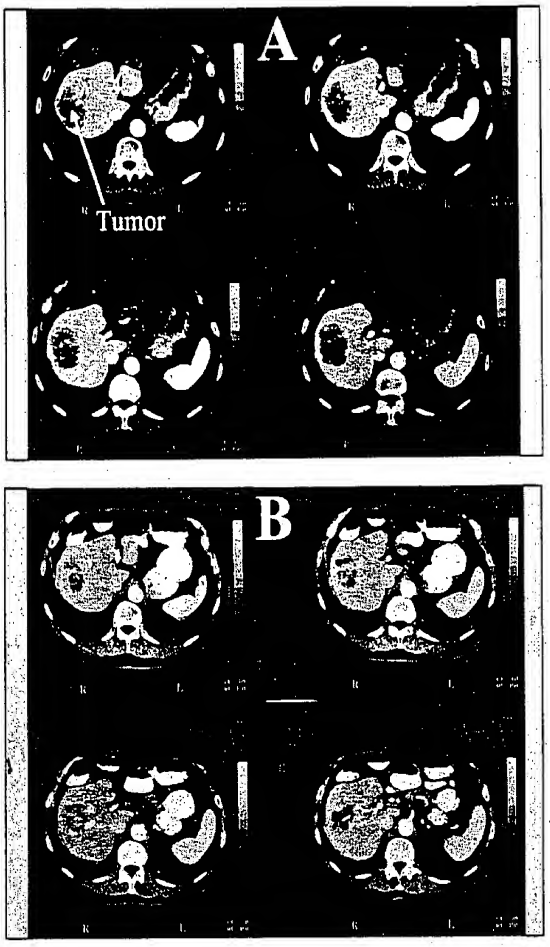

Fig 2. Partial response in a man with colon carcinoma. (A) Baseline CT scan demonstrating 10-cm liver metastasis. (B) CT scan at month 1 demonstrating the response.

Replacement of Tumor by Inflammatory Cells on Histologic Examination

During the course of PV701 therapy, one patient had tumor tissue removed for electron microscopic examination and other tissue analysis. This 46-year-old man, who had bulky peritoneal mesothelioma that had progressed after debulking surgery and intraperitoneal doxorubicin/cisplatin and IFN-γ, was enrolled at the 12/48/48 × 10^9 PFU/m^2 dose level in January 1999. During 30 monthly courses of PV701, he has remained free from progression, has no disease-related symptoms, experienced an improvement in performance status (to Eastern Cooperative Oncology Group 0), gained muscle mass, and retained a high level of physical activity. CT scans performed on a monthly basis have shown up to a 35% reduction in bidimensional measurable disease (270 cm^2 at study entry). Elective surgery to debulk disease 2 weeks after his last dose of the eighth course (in the eleventh month of PV701 administration) was unsuccessful. However, histologic examination showed a significant fraction of the tumor mass replaced by an active inflammatory process with edema in all sections of tumor (Fig 3A and 3B). This process consisted predominantly of plasma cells. Lymphoid follicles with germinal centers were also evident in the tumor, indicating an active immune reaction (Fig 3C). Electron microscopy revealed PV701 particles at tumor cell membranes (Fig 3D). The plasma cell infiltrate and secondary lymphoid follicles were not present in previous sections of tumor parenchyma taken before enrollment. A normal skin sample removed at the time of the patient's tumor excision did not show any

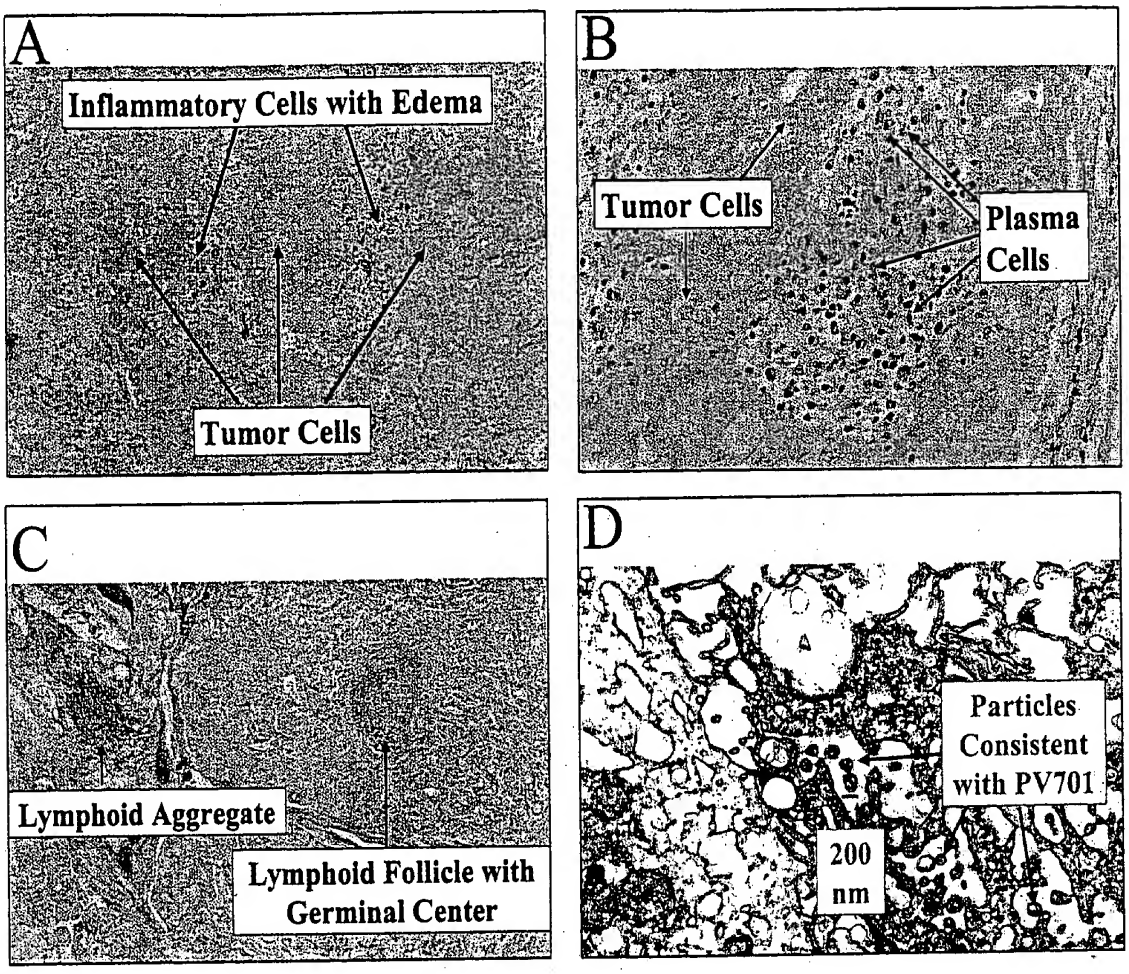

Fig 3. Microscopic tumor sections after 8 PV701 courses. Tumor parenchyma (H&E histologic stain) showing (A) inflammation/edema, (B) plasma cell infiltrate, and (C) lymphoid follicle/aggregate. (D) Electron micrograph shows particles consistent with PV701 budding from the tumor cell membrane.

evidence of inflammation. His serum neutralizing antibody titer had reached a plateau level of 1:2,560 at the time of the tissue examination.

In another case, a different pattern of tumor inflammation was seen. Autopsy sections from a patient who had advanced pleural mesothelioma and died of progressive disease (tumor obstruction of the inferior vena cava) were reviewed. Lung metastases displayed a mononuclear inflammatory infiltrate mainly at the periphery of the larger metastases and throughout the smaller tumor masses (Fig 4A and 4B). Also observed in the lung metastases were signs of tumor necrosis, including multifocal areas at the tumor periphery (Fig 4A). The portion of lung without tumor was free of any signs of inflammation (Fig 4C). A similar pattern was seen in his liver metastasis (Fig 4D), which showed mononuclear inflammatory cells infiltrating the tumor but not uninvolved liver distal from the tumor (Fig 4E). A diffuse mononuclear inflammatory infiltrate was also seen in a mesenteric metastasis (Fig 4F) but not in the adjacent normal tissue. No such inflammatory process was present in the original biopsy of the primary tumor preceding PV701 treatment. Unlike the previous case of the patient with peritoneal mesothelioma, there was no sign of tumor regression in this particular patient and no samples of tumor tissue were obtained for viral analysis.

DISCUSSION

Among the various clinical tests of replication-competent viruses[22-25,28,29] (Stojdl et al, manuscript submitted for publication), the phase I dose escalation study reported here is the first study in humans to determine an MTD for systemic (intravenous) administration. PV701, an oncolytic

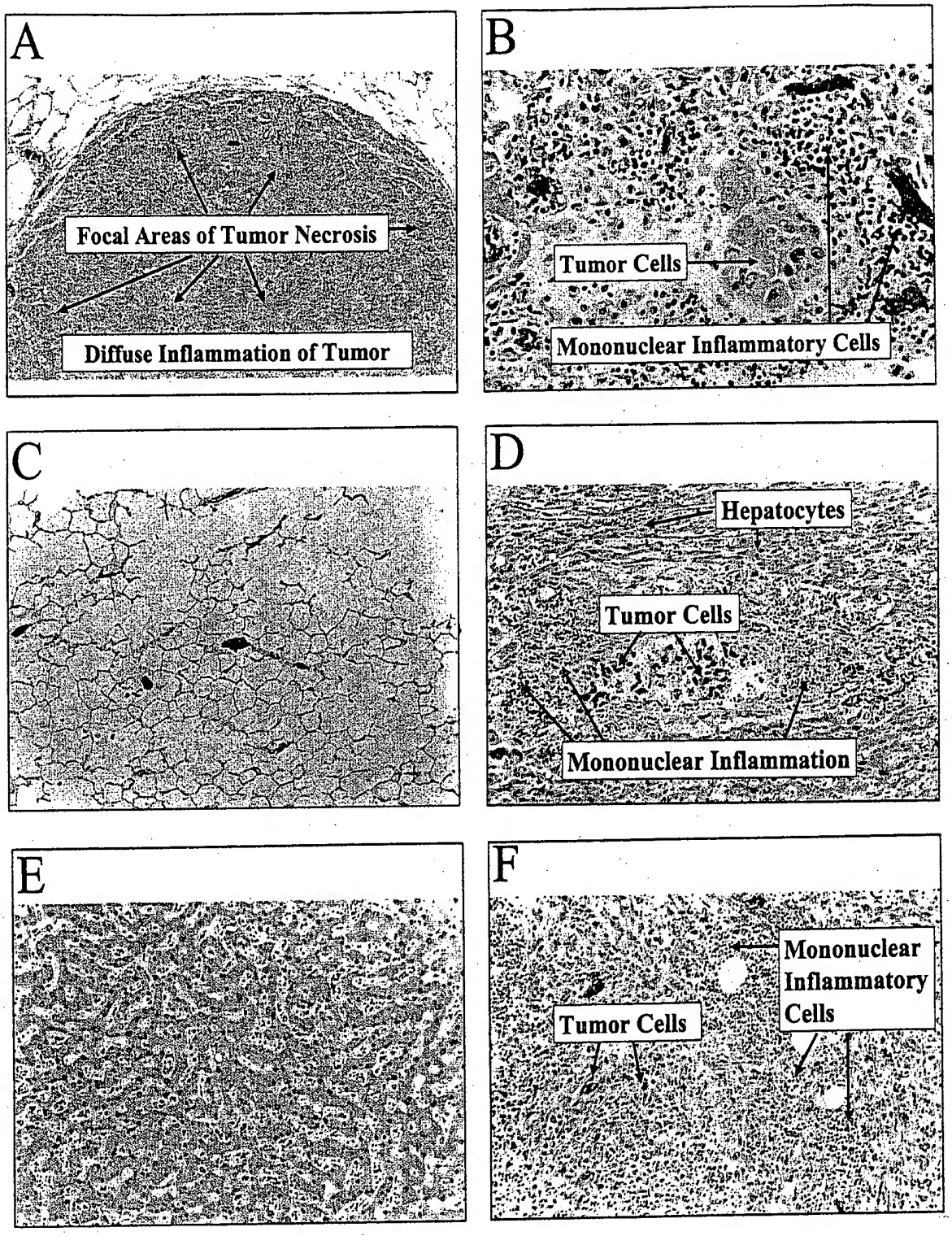

Fig 4. Histologic (H&E) tissue sections from a patient with pleural mesothelioma. (A, B) Lung metastasis showing inflammation and multifocal necrosis. (C) Lung uninvolved by tumor. (D) Liver metastasis showing inflammation. (E) Liver uninvolved by tumor. (F) Mesenteric metastasis showing inflammation.

strain of Newcastle disease virus was studied because it demonstrated preclinical activity against a wide range of human tumors in vitro and in vivo and is active by the intravenous route. Importantly, Newcastle disease virus has previously been shown to lack pathogenicity in humans after low-dose administration[3,8,20,21,30] mainly as a component of oncolysate tumor vaccines.[8,20,21] This phase I study characterized the toxicity profile for intravenous PV701 dosing and demonstrated the feasibility and potential benefit of systemic (intravenous) oncolytic virus administration.

As expected from previously published reports on Newcastle disease virus serologic surveys,[31,32] only one of the 32 patients tested was found to have neutralizing antibody before administration of the virus. After dosing with PV701, the majority (27 of 29) of patients tested developed varying levels of neutralizing antibody. Neutralizing antibody titers, even with multiple courses, reached a moderate plateau level of ~1:2,560, including a patient who received repeated cycles of PV701 for more than 18 months. Of potential clinical significance, signs of efficacy (eg, tumor regressions) were observed in patients after formation of these antibody titers.

Low levels of viral shedding were observed and found generally to be transient. Recovery of virus from sputum was rare, was of low level, occurred only after the first cycle of virus administration, and always cleared within a maximum of 14 days. Recovery of virus from urine after the first cycle of PV701 was more common but again did not persist, being cleared within 3 weeks. Transient viruria was observed less frequently after subsequent cycles but did occur despite the presence of neutralizing antibodies. Ultimately, the incidence of transient viruria diminished to zero among patients who received PV701 for seven or more cycles. Relative to the environmental impact of shedding on the most susceptible host species (chickens), the observed levels of PV701 shedding are orders of magnitude below the standard avian vaccine dose required for an antibody response.[33,34]

The low and transient viral shedding seen in this study may be part of the explanation for the lack of any observed human-to-human transmission seen with PV701. This finding is in agreement with data from other clinical trials using Newcastle disease virus[3] and with other human experience with the virus.[3,6]

The acute toxicity of PV701 principally consisted of flu-like symptoms (fever, chills, fatigue, headache, nausea, vomiting, and diarrhea) and dose-dependent hypotension that occurred 4 to 24 hours after PV701 dosing. Older patients (≥70 years) and those with anemia (hemoglobin < 11 g/dL) were found to be more likely to experience flu-like symptoms of greater severity. Acute toxic effects have been shown in animal models to be a result of Newcastle disease virus–induced release of proinflammatory cytokines, including type I IFNs and TNF-α.[35-39] Levels of type I IFN, IFN-γ, TNF-α, and IL-6 were elevated in patients in this study after the first dose of PV701, with levels first detected by 6 hours and peaking at hour 20. This pattern paralleled the time course of the flu-like symptoms (eg, fever was consistently noted between hours 4 and 20). Antipyretics and antidiarrheal agents reduced the incidence and severity of these toxicities.

Just as tachyphylaxis develops in mammals, including humans, with repeat IFN and TNF dosing,[40-42] a striking reduction in the incidence and severity of PV701-mediated acute toxicity on repeat dosing was observed (Table 4). This phenomenon, termed "desensitization," was first observed with PV701 in the rodent models and applies to effects on toxicity but not efficacy. In preclinical testing using human tumor xenografts in athymic mice, efficacy increased with repeat dosing and toxicity was markedly reduced. After intravenous administration of 3×10^8 PFU, mice tolerated subsequent intravenous doses of 1×10^{10} PFU, indicating at least a 10-fold increase in the MTD.[2] As fully predicted by these preclinical studies, this desensitization phenomenon allowed a 10-fold increase in the MTD observed in this trial with a first dose MTD of 12×10^9 PFU/m^2 and a second dose MTD of 120×10^9 PFU/m^2. The reduction in adverse event profile seen with the second PV701 dose compared with the first dose paralleled the reduction in serum cytokine levels seen after second PV701 dose, suggesting a causative role of proinflammatory cytokines in the clinical toxicity of PV701. As seen preclinically in both immunodeficient and immunocompetent mice, this desensitization phenomenon in patients does not depend on the development of antibodies to PV701 because it is seen as early as 2 days after the first PV701 dose (when antibodies are not detectable).

Desensitization also occurred with respect to transient drops in platelet and WBC counts. IFN and TNF-α are known to cause transient changes in blood cell counts as a result of margination.[40,43-47] A previous study by Merrigan et al,[30] using single doses (from 10^6 to 10^8 PFU/patient) of Newcastle disease virus, orders of magnitude below the doses tested in this trial, verified a dose-dependent induction of IFN in the serum of 17 patients along with fever and a transient leukopenia. In the present study, these transient hematologic changes were induced by the first dose of PV701. Leukocyte and platelet levels recovered during the first course of repeat dosing, even when doses were 10-fold higher than the first dose. Importantly, the leukopenias and thrombocytopenias were not correlated with signs of infection or bleeding, and the degree and rate of recovery did not

require therapeutic intervention. The lack of cumulative effects seems to be consistent with margination of leukocytes and platelets rather than a consumptive process.

There was no observed cumulative toxicity associated with prolonged repeated PV701 dosing including a total of 116 repeat courses given to a total of 39 patients. One patient has had more than 30 courses of PV701 with no evidence of an adverse effect on any organ system. Because of desensitization and the lack of cumulative toxicity, an overall dose intensification of more than 100-fold was achieved in this trial (Table 1).

Tumor site–specific inflammatory reactions were also seen. In this study, two patients with palpable tumors (colon cancer with a scalp metastasis and tongue cancer) developed inflammation and edema localized to the tumor sites. Histologic confirmation of tumor site–specific inflammation was obtained from representative patients. In one patient with metastatic pleural mesothelioma, tumor necrosis and a mononuclear cell inflammatory infiltrate were observed only at tumor sites without any involvement of normal tissue, including tissue adjacent to disease sites (Fig 4). Evidence of such tumor inflammation as seen in this trial raises a question about the determination of responses by traditional imaging criteria, especially in future phase II trials.

Tumor site–specific effects were also observed in patients with tumor involvement of the lung and liver. Oxygen desaturation, for example, was observed only in patients with pulmonary/pleural-based tumors (13 of 55 with involvement *v* zero of 24 without; $P < .01$). Significant elevation in liver enzymes was also limited, occurring only in patients with liver metastases (18 of 38 *v* zero of 41; $P < .01$). In addition, there was no evidence of generalized hepatocyte toxicity because total serum protein and clotting times remained comparable to baseline. Furthermore, the typical pattern of liver enzymes (elevated gamma glutamyltransferase and alkaline phosphatase disproportionate to transaminases; and AST > ALT) was indicative of pressure on the canaliculi and cholangioles from a space-occupying effect rather than hepatocellular damage from hepatitis.[48,49] Of cautionary note for future trials, patients with malignancy extensively replacing normal lung tissue, particularly if baseline pulmonary dysfunction exists, seem to be at risk for severe pulmonary toxicity. One such patient with preexisting compromised lung function died of respiratory failure. Severe edema and inflammation was found localized to the tumor-bearing lung along with thrombosis confined to the tumor vessels.

Response assessment was not the focus of this phase I study, especially because, in this dose escalation study, most patients received low, potentially suboptimal dose intensities (Table 1). However, 62 patients were assessable. Evidence of efficacy included progression-free survival from 4 to more than 30 months in 14 patients who had clear evidence of disease progression before initiation of PV701. Furthermore, two radiographic objective responses (complete response and partial response) were documented and seven other patients had measurable tumor reductions, although not to the degree of a partial response. A 46-year-old man with advanced peritoneal mesothelioma unresponsive to intraperitoneal chemotherapy, with bulky disease (four 8- to 10-cm masses with total bidimensional measurable disease of 270 cm^2) at baseline, has received more than 30 courses of PV701, has maintained an improved performance status (Eastern Cooperative Oncology Group 0), has had a radiographic minor response (of 35% tumor regression), and has experienced no cumulative toxicity. Evidence of a direct oncolytic effect of PV701 in this patient was found on biopsy after 11 months of PV701 administration. PV701 particles were observed budding from tumor cell membranes, and the tumor mass was extensively filled with mononuclear inflammatory cells (especially plasma cells) replacing tumor, indicating that PV701 had gained access to the tumor and was replicating there despite the presence of serum neutralizing antibody. In comparison to this patient with one of the largest tumor burdens enrolled onto the study, the patient with the smallest tumor burden (1.5 cm, tonsillar cancer) experienced a complete radiographic response after three doses of PV701.

Collectively, these observations support the concept that systemic therapy with the replication-competent virus PV701 can provide a novel and potentially important therapy for patients with solid tumors, including those unresponsive to standard therapy. Moreover, long-term intravenous virus therapy seems to be feasible in humans and may play an important role in the treatment of solid tumors. Additional clinical studies of PV701 have begun.

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